

## The Variation in Karyotypes of *Brachytarsophrys* from China with a Discussion of the Classification of the Genus

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**Abstract.** -Different karyotypic forms of *Brachytarsophrys* occur in populations from Jingdong and Dayao, Yunnan. These forms, along with morphological evidence, suggest that *Brachytarsophrys* should be divided into two different species. The Jingdong population is identified as *B. feae* (previously *Megophrys feae*). The Dayao population is a new species, resembling in morphology, *B. carinensis* which occurs in south Burma and Thailand. The Dayao population is geographically far removed from *B. carinensis*. A valid sister species, *B. feae*, is found between the distribution of the Dayao population and *B. carinensis*. The new species is found in a different climatic zone and in different habitats than *B. carinensis*. *B. intermedius* in south Vietnam probably is not a valid species because it is more similar to *B. carinensis* in morphology and shares the same climatic zone and habitats.

**Key words:** *Brachytarsophrys*, *B. platyparictus* sp. nov., *B. feae*, karyotype, biogeography, classification

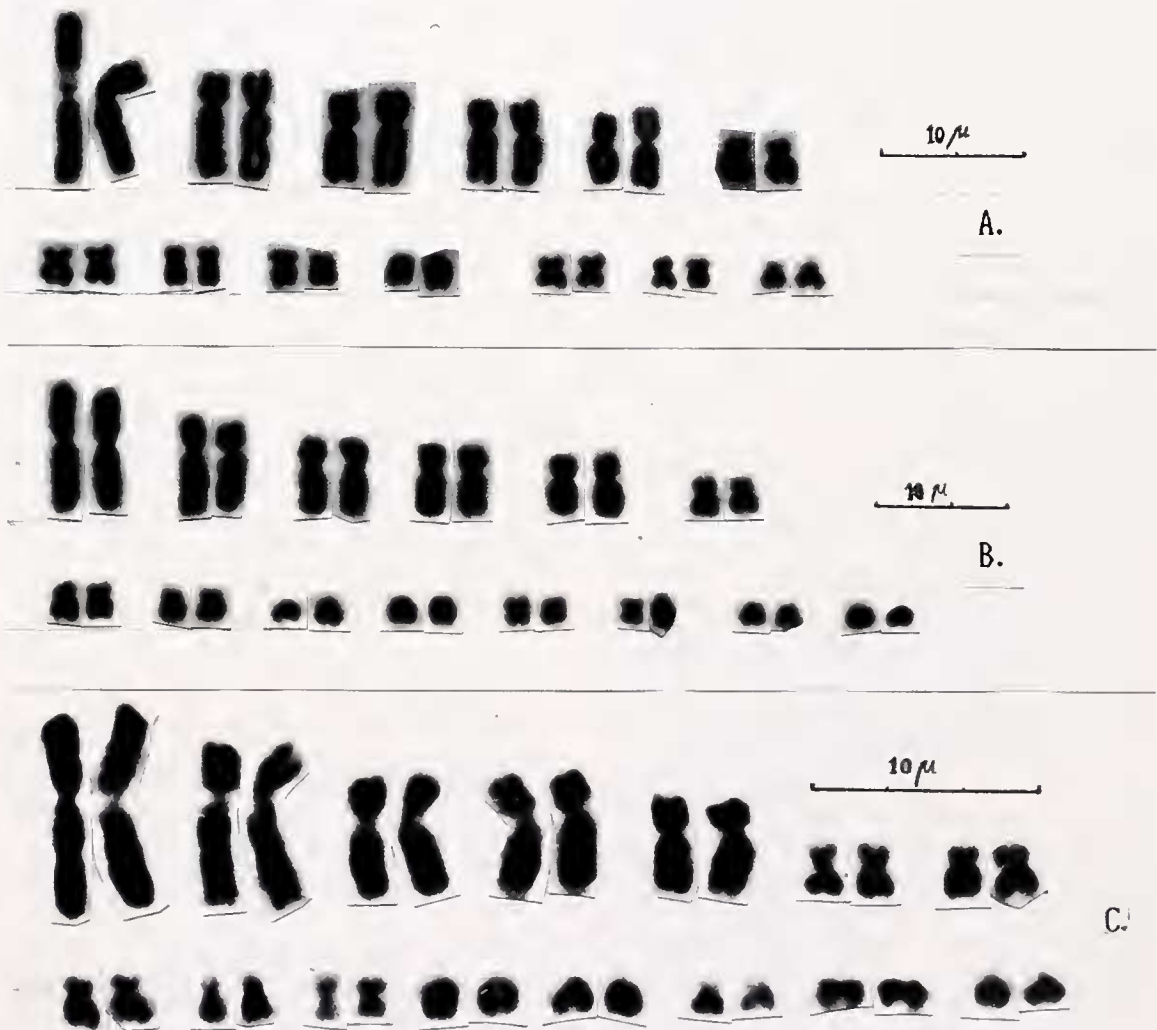


Figure 1. The karyotypes of *Brachytarsophrys* of China. A: Jingdong population; B-C: Dayao population.

### Introduction

*Brachytarsophrys* in China were considered to be only *B. carinensis* until Ye and Fei (1992) described a second species, *B. feae*. Our studies of cytology and morphology agree with Ye and Fei that *Brachytarsophrys* in China belong to two different species. The Jingdong population we studied belongs to *B. feae*. We do not agree with them with respect to the Dayao population, which they identified as *B. carinensis*.

### Materials and Methods

Specimens used in cytological research were collected respectively from Jingdong, Yunnan in June, 1990 and May, 1991, and Dayao, Yunnan in May, 1990. All specimens were taken to the laboratory before processing. Karyotypes were prepared using the method of colchicine-hypotonic-air drying: specimens were injected intraperitoneally with colchicine at a dosage of 20 µg/g body weight for about 24-36 hours, then the femur and tibia were cut off at their two ends. The marrow cells were washed out with 1% tri-sodium citrate solution, ground, then placed in a hypotonic solution of 0.64% KCl and fixed in 3:1 solution of methanol:acetic acid for two periods of 20 minutes each. Slides were air-dried and stained with 10% Giemsa PBS for about 15 minutes. Specimens from middle, southern, southwestern, northwestern and northern Yunnan were examined and some from Jingdong, Menglian, Tengchong, and Xishuanbanna were dissected.

### Cytological Results

The karyotypic form of the specimens from Jingdong is 2n=26, 5 large and 8 small pairs, Nos. 1, 3, 4, 5, 7, 8, 11, are M (metacentric); Nos. 2, 6, 9, and 13 are SM (submetacentric); only No. 10 is T (telocentric). The NF (fundamental number) is 50. One secondary constriction is found in the near base of the short arm of No. 1; no satellite was found. The karyotypic form of the specimens from Dayao is mostly 2n=28, 5 large and 9 small pairs, Nos. 1, 3, 4, 5, 6, 7, 11, and 12 are M; Nos. 2 and 8 are SM and Nos. 9, 10, 13, and 14 are T chromosomes, NF=48. No satellite or secondary constrictions were found. We also found a third karyotypic form with 2n=30 in Dayao specimens, with 5 large and 10 small pairs, Nos. 2, 7, and 8 are SM, Nos 9 and 13 are ST and Nos. 11, 12, 14, and 15 are T; the rest are SM chromosomes, NF=48. No secondary constrictions and satellites were found. A few speci-

Table 1. Comparison of karyotypes of *Brachytarsophrys* from different localities in China.

Locality	2n	NF	Large Group					Small Group									
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Jingdong	26	50	M*	SM	M	M	M	SM	M	M	T	M	M	M	SM		
Dayao	28	48	M	SM	M	M	M	M	SM	T	T	M	M	T			
Dayao	30	48	M	SM	M	M	M	M	SM	ST	M	T	T	ST	T		
Sichuan	26	44	M	SM	M	M	M	M	M	T	M	T	T	T			(ST)

mens had 2n=26, as reported by Tan et al. (1987) for specimens from Miyi and Huili, Sichuan (omitted from this article, Table 1).

## Discussion of Karyotypes

Table 1 shows that the large chromosomes are similar in the three populations examined, but the small chromosomes vary. The karyotype of the specimens from Dayao is closer to that from Sichuan than that from Jingdong. The karyotype of the population from Jingdong has  $2n=26$ , NF 50, an obvious secondary constriction in 1p, only 1 telocentric pair (No. 10) of chromosomes, 3 pairs of SM chromosomes (Nos. 6, 9, 13). Small chromosomes are more numerous in this population than in the other two populations. The karyotype of the Dayao population is  $2n=28$ , NF=48, no secondary constriction was found, only 1 SM chromosome (No. 8), and 4 small T chromosomes (No. 9, 10, 13, 14). This is more than the Jingdong, Sichuan population karyotypes which were mainly  $2n=26$ , NF=44, 1 secondary constriction in each of two T chromosomes (No. 9, 11), no SM chromosomes, and 4 T chromosomes (No. 9, 11, 12, 13) (Tan, et al., 1987). Because of these differences in karyotype, the populations of Jingdong and Dayao should be recognized as two different species.

## Morphological Results

The specimens from northeastern Yunnan (Lijiang, Yangbi, Bianchuan, Dayao, Yuxi, Kunming, Shuangbai, Shiping and Xinping), from Sichuan (Miyi, Huili), and from Guizhou (Leishan, Anlong) (Wu et al., 1986) are probably the same as those from Guangxi (Longsheng, Jinxui), from Hunan (Yizhang), and from Jiangxi (Jinganshan). The toes are obviously webbed and the web may reach or exceed 1/3 of the toes. The tops of the skulls in specimens from middle and northern Yunnan are very flat. Specimens from southwestern Yunnan (Jingdong, Meglian, Xishuanbanna, Tengchong, Yongde and Longchuan, near the type locality of *Megophrys feae*) are nearly free of webbing or with a mere rudiment of a web. The tops of their skulls are obviously depressed. These differences suggest that these forms be recognized as two different species.

Bourret (1942) and Taylor (1962) described *B. carinensis* (previously *Megophrys carinensis*) as follows: eyelid with two to four pointed tubercles near edge, vomerine teeth normally present, heel reaches to shoulder or jaw-angle, skin with bony deposits above head and anterior part of body, a vocal sac, paired elongate glandular folds of back, male 123 mm and female 150 mm, and toes about 1/3 webbed. It is distributed in Thailand (Chang Mai, Lampang provinces and Mergui) and south Burma (type locality is Karen Hills in southwestern Burma).

*B. feae* (*M. feae*) is characterized by eyelids with small tubercles, one much more elongate than the others, no longitudinal glandular folds on dorsum, a vocal sac, tibiotarsal articulation which reaches near jaw-angle, bony deposits on head, length 106 mm, head depressed, and toes free or with a mere rudiment of web. It is distributed in north Burma and the type locality is Kakhien Hills, Burma which is near Longchuan and Tengchong, Yunnan, China.

The population of southwestern Yunnan is here assigned to *B. feae* and is not *B. carinensis*. The populations of northeastern Yunnan and Sichuan are similar to *B. carinensis*, but lack paired glandular folds.

## Discussion

The populations from north and middle Yunnan, including the Dayao population, are similar to *B. carinensis* in possessing eyelids and toe-webs, but they obviously lack paired elongate granular folds on the back that are present in *B. carinensis* (Bourret, 1942; Taylor, 1962). Also these Yunnan populations are geographically widely separated from *B. carinensis* and are found in different habitats and climate zones. *B. carinensis* occurs in tropical habitats whereas populations of northeastern Yunnan occur in temperate zones, and are separated from *B. carinensis* by a valid sister species, *B. feae*.

Physiological characteristics limit amphibian ability to disperse. Distributions should be continuous and presently discontinuous distributions should be vestiges of a once continuous range. The distribution of a species should be continuous, and even when populations are separated by a valid sister species or different climatic zones, such populations should be widely separated. We think that the population of northeastern Yunnan, Sichuan and nearby localities should be a new species and not recognized as *B. carinensis*.

*B. intermedius* in south Vietnam (Lang Bian) has paired elongate folds; it is similar to *B. carinensis*, and inhabits the same climatic zone (tropical). It is probably a population of *B. carinensis*.

Karyotypic and morphological evidence and geographical distribution (Liu and Hu, 1961; Yang et al., 1991; Wu et al., 1986; Tian and Jiang, 1986), suggest that the population from Jingdong (including those of southwestern Yunnan) is *B. feae*. The populations from Dayao (including northeastern Yunnan, Sichuan, Guizhou, Guangxi, Hunan and Jiangxi) is a new species and not a population of *B. carinensis*. *B. carinensis* is distributed in southwestern Burma (type locality) and north Thailand. *Megophrys intermedius*

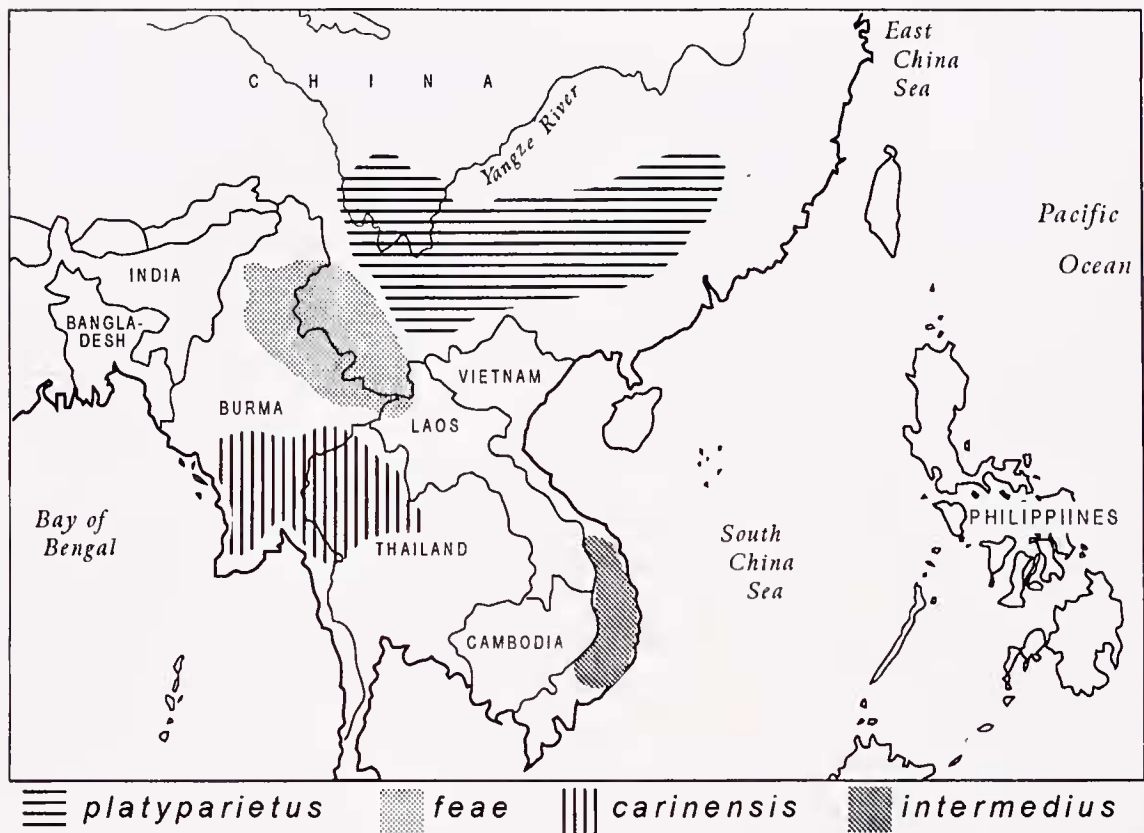


Figure 2. The distribution of the genus *Brachytarsophrys*.

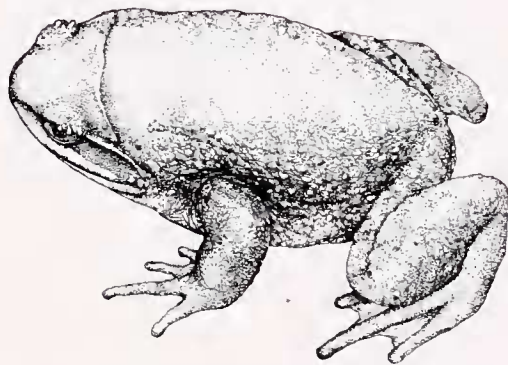


Figure 3. *Brachytarsophrys platyparietus* sp. nov. Holotype (KIZ 90275), adult male X 1.

Smith (1921) in south Vietnam (Annam) is a population of *B. carinensis*. We recognize three species in the genus *Brachytarsophrys*: *B. carinensis* in south-western Burma, Thailand and south Vietnam; *B. feae* in north Burma and southwestern Yunnan; and a new species in northeastern Yunnan, southern Sichuan, Guizhou, Guangxi, Hunan and Jiangxi (Fig. 2).

### Taxonomic Account

*Brachytarsophrys platyparietus* sp. nov. (Fig. 3)

**Holotype:** KIZ 90275, adult male, collected from Duo-Di-He, San-Tai, Da-Yao, Yunnan, China, altitude 2100 meters.

**Paratypes:** 1 adult male (KIZ 90274) and adult female (KIZ 90276), from the same locality as the holotype.

**Diagnosis:** Body very large, male may reach 116 mm from snout to vent, female 131 mm; eyelids with three circular tubercles in a line, the middle longest, and not very sharp; tympanum hidden; arched diagonal fold from eyelid to above arm insertion; toes widely fringed and obviously webbed (at least extending to 1/3 toes); the top of skull obviously very flat; no longitudinal granular folds on the body side; no subarticular tubercles. The karyotype with  $2n=28$ , few are 26 or 30; 5 pairs of large chromosomes, always with 4 pairs of small telocentric chromosomes, no secondary constriction in the No. 1 chromosome.

**Description of Holotype:** Head large, flat and wide, length nine-tenths of width; snout obtuse, very short.

its length in front of the level of eyes 12 mm; distance between nostrils 11 mm; canthus rostralis not very sharp; loreal region only slightly diagonal; eyelid elevated, projecting, bearing three elongate and circular tubercles in a line, among which the middle one is longer than the other two, and with much smaller tubercles (3-4) behind them; tongue broad, nearly round, mostly free behind, free on sides, one notch in front; chaoanae partly concealed by maxillary shelves; vomerine teeth present; arm rather long in proportion to leg; first finger shorter than second; third longest; no web between fingers and no fringe; legs relatively short, tibiotarsal articulation reaching to back of skull; toes about one-third (or more) webbed and widely fringed; a large flat inner metatarsal tubercle, as long as first toe; no tarsal fold; digits without widened tips; heels separated by about 20 mm when legs are folded; no subarticular tubercles.

Skin above generally smooth, but laterally with numerous flat tubercles; a strong fold from edge of eyelid to above arm insertion on each side, the folds are arched; back without pair of curving ridges from head to shoulders, and without any dorso-lateral ridges on dorsal side and above groin; no paired conical symmetrical tubercles in front of shoulders; no sharp ridges and tubercles on sides; tibia without any ridges. Body length 113 mm, head length 47 mm, width of head 57 mm.

**Variation:** One female (KIZ 90276) with body length 131 mm, head length 52.5 mm, head width 61 mm.

**Distribution:** Yunnan (Yangbi, Lijiang, Bianchuan, Dayao, Kunming, Yuxi); Sichuan (Miyi, Huili, Duko, Muli, Jiulong); Guizhou (Anlong, Leishan); Guangxi (Longshen, Jinxiu); Hunan (Yichang); and Jiangxi (Jingganshan).

**Comparisons:** *B. feae* is only very narrowly fringed and not webbed in the toes. The top of the skull is obviously depressed and the eyelid tubercle is very long and sharp. The karyotype is  $2n=26$  with five large pairs, only one pair of small telocentric chromo-

somes, and a secondary constriction in the short arm of the No. 1 chromosome. *B. carinensis* has webbed toes, obvious granular folds on the back and above the groin. The eyelids have three sharp pointed flattened tubercles. *B. intermedius* from south Vietnam is very similar to *B. carinensis*. It also has longitudinal granular folds on the back. We suggest that it should be considered a synonym of *B. carinensis*.

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